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Suanzaorentang, an Anxiolytic Chinese Medicine, Affects the Central Adrenergic and Serotonergic Systems in Rats

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ABSTRACT

Previously, we found that the ancient Chinese remedy of Suanzaorentang was a promising anxiolytic drug (Chen and Hsieh, 1985; Chen and Hsieh, 1985a). We also found that Suanzaorentang decreased the turnover rate of central monoamines and central catecholaminergic activity (Hsieh, *et al.*, 1986).

In this study, we found that (1) 5-hydroxytryptophan (5-HTP) induced decrease in locomotor activity was significantly antagonized by Suanzaorentang, (2) p-chlorophenylalanine (p-CPA) induced increase in locomotor activity was significantly inhibited by Suanzaorentang, (3) Suanzaorentang had no significant effects on baclofen, muscimol, aminooxyacetic acid (AOAA) and thiosemicarbazide induced changes in locomotor activity, (4) Suanzaorentang significantly decreased vanillylmandelic acid (VMA) in striatum and hippocampus, homovanillic acid (HVA) in hippocampus and 5-hydroxyindol acetic acid (5-HIAA) in striatum and hypothalamus, (5) Suanzaorentang significantly reversed the α-methyltyrosine (α-MT) produced decrease in DA concentrations in striatum and hippocampus, and (6) Suanzaorentang significantly reversed the p-CPA produced decrease in 5-HT concentrations on amygdala. These facts implied that Suanzaorentang might decrease the serotonergic activity but have no significant effect on GABAergic activity. The main locus of action might be in the limbic system.

Key words: Suanzaorentang, adrenergic, serotonergic

Introduction

Due to the ever-increasing tension in modern life, anxiety becomes a common disorder leading people to seek help. Benzodiazepines are the most commonly used anxiolytic agents because of their safety, efficacy, and low incidence of tolerance. However, benzodiazepines still have some adverse effects: neocortical depression characterized by tiredness, drowsiness and a feeling of detachment, hostility, dependence and withdrawal syndrome characterized by agitation. Looking for new anxiolytic agents with a more specific action and fewer adverse effects is a challenging problem to pharmacomedical staff.

The search for "ataractics" in China may be traced back to about 2000 years ago when the formula of Suanzaorentang was described in the medical book called Chinkuei-yao-lueh (Chang, 115 B.C.). In our pilot clinical trials of this formula (Chen and Hsieh, 1985; 1985a), we found that it was a promising anxiolytic agent. To understand the mechanisms of Suanzaorentang, effects of Suanzaorentang on behavioral changes and central monoamines had been studied in rats and it was found that Suanzaorentang might decrease the turnover rate of central monoamines and catecholaminergic activity (Hsieh *et al.*, 1986).

The purpose of the present study was to try to find the central locus where monoamines were mainly affected and to study the effects of Suanzaorentang on serotonergic and GABAergic systems.

Materials and Methods

Preparation of Suanzaorentang

The remedy of Suanzaorentang is composed of 5 components: Zizyphi semen, Poria, Ligustici Chuanxiong Rhizoma, Anemarrhenae Rhizoma and Glycyrrhizae Radix. The method of preparation of Suanzaorentang has been described in our previous paper (Chen and Hsieh, 1985). When given to rats, Suanzaorentang was suspended in 0.5% CMC (carboxymethylcellulose).

Animals

Male Sprague-Dawley rats, weighing between 190 and 230 g were used in the studies of locomotor activity. Male Sprague-Dawley rats, weighing between 260 and 300 g, were used in the studies of the concentration of monoamines-metabolites in different parts of the brain. Rats

were maintained and fed in the same way as that described in our previous paper (Hsieh and Chen, 1986).

Locomotor Activity Measurements

The locomotor activity of individual rats was measured with the MK-Animex activity meter (Model SE, Muromachi Kikai Co., Japan). The method used to measure the locomotor activity has been described in our previous paper (Hsieh and Chen, 1986).

Suanzaorentang was given p.o., 5-HTP, p-CPA, baclofen, muscimol HBr, AOAA and thiosemicarbazide were given i.p.

Suanzaorentang was given at the dose of 3.0 g/kg (calculated as the weight of crude herb). 5-HTP (50 mg/kg) and thiosemicarbazide (0.5 mg/kg) were given simultaneously with Suanzaorentang. p-CPA (200 mg/kg) was given 24 hours before Suanzaorentang. Baclofen (0.5 mg/kg) and muscimol HBr (0.5 mg/kg) were given 10 minutes before Suanzaorentang. AOAA (1 mg/kg) was given 3 hours before Suanzaorentang. Control rats were given 0.5% CMC p.o.

Determination of the Concentrations of Monoamines-Metabolites in Different Parts of the Rat Brain

The rats were decapitated and the whole brain was removed and separated into the following 8 parts according to the method of Glowinski (1966): cortex, striatum, hippocampus, midbrain, hypothalamus, amygdala, medulla oblongata and cerebellum. The concentrations of serotonin (5-HT), dopamine (DA), norepinephrine (NE) and their metabolites were measured by High Performance Liquid Chromatography (HPLC) with Electrochemical Detection (Gunther, 1982; Lackovic *et al.*, 1981; Shibuya *et al.*, 1982). HPLC Model M-45 (Waters Associates), Data Model M-730 (Waters Associates) and Electrochemical Detector Model LC-4B (Bioanalytical systems) were used.

NA, DA and 5-HT Turnover in Different Parts of the Rat Brain

α-MT (250 mg/kg, i.p.) or p-CPA was given three or twenty-three hours before Suanzaorentang (3 g/kg, p.o.) respectively. One hour after giving Suanzaorentang, the rats were decapitated and the whole brain was removed and separated into the 8 parts as described before. In another 5 rats only α-MT or p-CPA was given.

Statistical Analysis

When comparisons were made among 3 or more sets of data, results were analyzed statistically using analysis of variance to decide whether there was any significant difference between sets of data. If the difference was significant, then the comparisons were made between each experimental group and the control group using the Studentized range test:

$$|\bar{X}n - \bar{X}o| = C\sqrt{\frac{MSE}{n}},$$

where $\bar{X}n$ is the mean value of each experiment group,
$\bar{X}o$ is the mean value of the control group,
C is the upper 5% value in the Studentized range,
MSE is the mean square error, and
n is the number of data in each group.

A p value ≤ 0.05 was required for statistical significance.

When comprisons were made between 2 sets of data, the unpaired Student's test was used. A p value $\leq$ 0.05 by two-tailed analysis was required for statistical significance.

Results

Effects on Locomotor Activity

As shown in Fig. 1, 5-HTP induced decrease in locomotor activity was significantly antagonized ($p < 0.05$) by Suanzaorentang between 60 minutes and 180 minutes after giving 5-HTP and Suanzaorentang simultaneously.

As shown in Fig. 2, p-CPA induced increase in locomotor activity was significantly inhibited ($p < 0.05$) by Suanzaorentang from 60 minutes to 120 minutes after giving p-CPA and Suanzaorentang simultaneously.

Suanzaorentang had no significant effects on baclofen, muscimol, AOAA and thiosemicarbazide induced changes in locomotor activity.

Effects on Monoamines-Metabolites in Different Parts of the Rat Brain

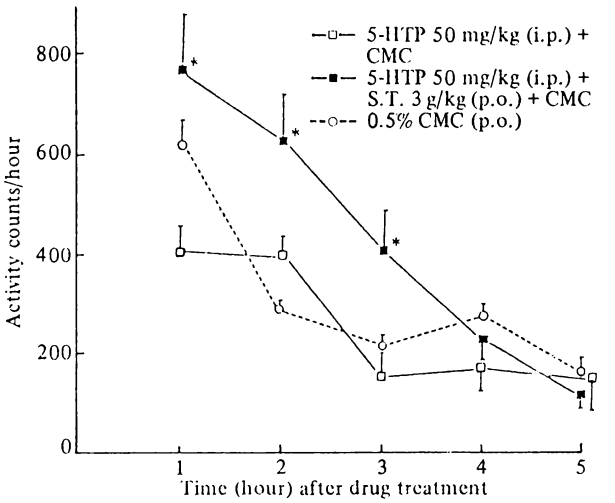

Fig. 1. Effects of Suanzaorentang on changes in locomotor activity produced by 5-HTP in rats. 5-HTP + S.T. + 0.5% CMC is compared to 5-HTP + 0.5% CMC. Each point represents the mean of 10 independent observations. The vertical bars designate the S.E.s'.
* $p < 0.05$ (compared to 5-HTP).

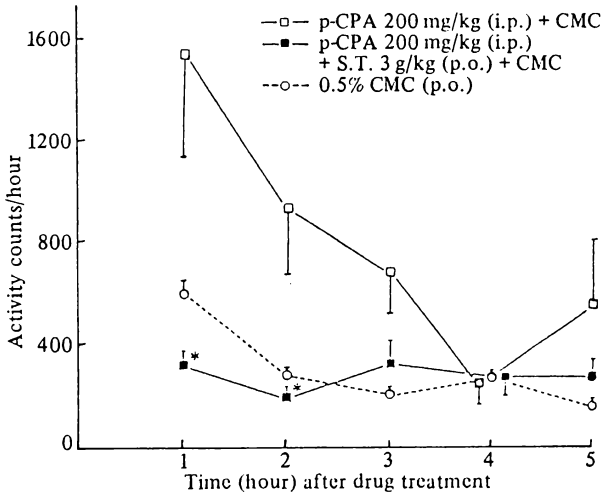

Fig. 2. Effects of Suanzaorentang on changes in locomotor activity produced by p-CPA in rats. p-CPA + S.T. + 0.5% CMC is compared to p-CPA + 0.5% CMC. Each point represents the mean of 10 independent observations. The vertical bars designate the S.E.s'.
* $p < 0.05$ (compared to p-CPA).

As shown in Table 1, Suanzaorentang had no significant effects on tissue concentrations of monoamines (NA, DA and 5-HT) in the 8 parts of the rat brain. Suanzaorentang significantly decreased the level of VMA in the striatum ($p < 0.01$) and the hippocampus ($p < 0.05$), HVA in the hippocampus ($p < 0.01$), 5-HIAA in the striatum ($p < 0.01$) and the hypothalamus ($p < 0.05$).

Effects of Suanzaorentang on NA, DA and 5-HT Turnover

As shown in Table 2, Suanzaorentang slightly but not significantly reversed the α-MT produced decrease in NA concentrations.

As shown in Table 3, Suanzaorentang significantly ($p < 0.05$) reversed the α-MT produced decrease in DA concentrations in the striatum and the hippocampus.

As shown in Table 4, Suanzaorentang significantly ($p < 0.05$) reversed the p-CPA produced decrease in 5-HT concentrations in the amygdala.

Discussion

5-HTP, a precursor of serotonin, can increase the central serotonin level with a subsequent decrease in locomotor activity (Everett, 1974). Suanzaorentang can

Table 1. Effects of Suanzaorentang (Alcohol Extracts) on Monoamines and Metabolites in Various Regions of the Rat Brain

		Cortex	Striatum	Hippocampus	Midbrain	Hypothalamus	Amygdala	Medulla oblongata	Cerebellum
NA	Control	1150±130	1870±170	1550±370	1420±200	1460±120	1680±170	1450±150	1390±50
	S.T.	1210±90	1700±130	1900±180	1710±480	1530±90	1520±70	1540±160	1520±130
DA	Control	1160±230	1620±130	1490±300	1430±350	1600±230	1460±190	1350±50	1410±100
	S.T.	1200±110	1490±60	1370±100	1450±70	1640±230	1350±130	1250±180	1490±90
5-HT	Control	1370±80	1620±200	1360±120	1640±140	1500±180	1430±250	1460±100	1190±160
	S.T.	1170±70	1540±90	1330±70	1490±70	1490±100	1460±250	1460±250	1280±150
VMA	Control	427±28	1530±100	1460±140	575±83	582±43	526±66	533±61	505±43
	S.T.	420±55	935±75**	882±141*	559±97	638±65	491±53	501±40	549±61
HVA	Control	940±71	916±67	1370±100	635±84	537±75	545±61	542±67	501±59
	S.T.	756±57	918±108	805±92**	598±70	540±68	569±66	549±25	477±43
5-HIAA	Control	562±57	1250±100	1500±40	740±88	688±99	575±69	530±49	502±54
	S.T.	419±29	639±94**	1440±100	671±52	416±33*	596±82	510±59	493±38

(1) Mean ± S.E. (n=5)
(2) *$p < 0.05$, **$p < 0.01$ as analyzed by unpaired comparison of Student's t-test
(3) control: 0.5% CMC, S.T.: Suanzaorentang alcohol extracts 3.0 g/kg (p.o.)
(4) S.T. is compared to control (0.5% CMC)

Table 2. Effects of Suanzaorentang (Alcohol Extracts) on Noradrenaline Turnover in Rat Brain

Parts of brain / NA	Cortex	Striatum	Hippocampus	Midbrain	Hypothalamus	Amygdala	Medulla oblongata	Cerebellum
Control (0.5% CMC)	1150±130	1870±170	1550±370	1420±200	1460±120	1680±170	1450±150	1390±50
α-methyl-p-tyrosine (α-MT) 250 mg/kg i.p. + CMC	963±82	996±45	992±84	1110±110	896±100	1030±120	1020±90	970±104
α-MT 250 mg/kg i.p. + S.T. 3 g/kg p.o. + CMC	1070±130	1150±120	1070±60	1200±110	992±151	1130±90	1110±150	1050±150

(1) Mean ± S.E. (n=5)
(2) NA concentrations are in ng/g wet weight
(3) No significant difference between α-MT + CMC and α-MT + S.T. + CMC

Table 3. Effects of Suanzaorentang (Alcohol Extracts) on Dopamine Turnover in Rat Brain

DA \ Parts of brain	Cortex	Striatum	Hippocampus	Midbrain	Hypothalamus	Amygdala	Medulla oblongata	Cerebellum
Control (0.5% CMC)	1160±230	1620±130	1490±300	1430±350	1600±230	1460±190	1350±50	1410±110
α-methyl-p-tyrosine (α-MT) 250 mg/kg i.p. + CMC	834±77	971±74	737±75	1030±110	1050±140	1020±100	929±120	930±101
αMT 250 mg/kg i.p. + S.T. 3 g/kg p.o. + CMC	991±109	1370±140*	1170±120*	1050±110	1070±100	1190±160	973±86	1020±130

(1) Mean ± S.E. (n=5)
(2) DA concentrations are in ng/g wet weight
(3) *$p < 0.05$ as analyzed by unpaired comparison of Student's t-test
(4) α-MT + S.T. + CMC is compared to α-MT + CMC

Table 4. Effects of Suanzaorentang (Alcohol Extracts) on 5-hydroxytryptamine (5-HT) Turnover in Rat Brain

5-HT \ Parts of brain	Cortex	Striatum	Hippocampus	Midbrain	Hypothalamus	Amygdala	Medulla oblongata	Cerebellum
Control (0.5% CMC)	1370±80	1620±200	1360±120	1640±140	1500±180	1430±250	1460±100	1190±160
p-chlorophenylalanine (PCPA) 200 mg/kg i.p. + CMC	892±107	1030±110	870±77	1140±90	1060±140	895±120	1020±70	893±130
PCPA 200 mg/kg i.p. + S.T. 3 g/kg p.o. + CMC	1010±80	1170±100	913±116	1170±100	1230±120	1330±60*	1130±90	950±84

(1) Mean ± S.E. (n=5)
(2) 5-HT concentrations are in ng/g wet weight
(3) *$p < 0.05$ as analyzed by unpaired comparison of Student's t-test
(4) p-CPA + S.T. + CMC is compared to P-CPA + CMC

reverse the depressed locomotor activity induced by 5-HTP. This fact implied that Suanzaorentang had an anti-serotonergic effect. p-CPA, a serotonin synthesis inhibitor, can cause catecholamine arousal with a subsequent increase in locomotor activity (Mabry and Campbell, 1973). Suanzaorentang can depress the increased locomotor activity induced by p-CPA. This fact implied that Suanzaorentang could decrease the catecholaminergic activity.

Baclofen (Wachtel *et al.*, 1978) and muscimol (Beaumont *et al.*, 1978) are GABA receptor agonists and decrease the locomotor activity, AOAA is a GABA-transaminase inhibitor that can increase the GABA level in the brain and decrease the locomotor activity (Wallach, 1961). Thiosemicarbazide, a glutamate decarboxylase inhibitor, can impair (GABA synthesis and cause convulsions. Therefore locomotor activity is decreased by thiosemicarbazide (Bell and Anderson, 1972). Suanzaorentang did not significantly alter the locomotor activity changes induced by baclofen, muscimol, AOAA and thiosemicarbazide. These facts might imply that Suanzaorentang had no significant effect on the central GABAergic system.

Anxiety is thought to be associated with the central catecholaminergic and serotonergic system (Rudolf, 1982). The muscular relaxant and ataxic adverse effects of benzodiazepines were thought to be associated with the GABAergic system (Hsieh *et al.*, 1984; Fielding and Hoffman, 1979). From this study and our previous study (Hsieh and Chen, 1986), we found that Suanzaorentang could reduce catecholaminergic and serotonergic activity but had no significant effect on the GABAergic system. These facts might explain why clinically Suanzaorentang had more specific anxiolytic effect but without the adverse effects of muscular weakness and ataxia (Chen and Hsieh, 1985a).

From the studies of monoamines-metabolites in 8 different parts of the rat brain, we found that Suanzaorentang could specifically decrease monoamine turnovers in the striatum, hippocampus, hypothalamus and amygdala, parts of the limbic system which are the main locus of emotional reactivity (Ganong, 1974; Hamilton, 1974). So, the anxiolytic mechanism of Suanzaorentang might be associated with its effects on decreasing the monoamine turnovers on the limbic system.

The anxiolytic effects of benzodiazepines are also thought to be associated with their being able to decrease monoamine turnovers (Fuxe *et al.*, 1975). However, the main locus of benzodiazepine action may be in the midbrain-hindbrain (Stein *et al.*, 1975).

In conclusion, the mechanism of Suanzaorentang may be related to its effects on the catecholaminergic and serotonergic systems. It has no significant effect on the GABAergic system. And the main locus of action may be on the limbic system. The mechanisms of action of Suanzaorentang are not quite the same as those of benzodiazepines.

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References

Beaumont, K., W.S. Chilton, H.I. Yamamura and S.J. Enna. (1978) Muscimol binding in rat brain: association with synaptic GABA receptors. *Brain Res.*, **148**, 153-162.

Bell, J.A. and E. Anderson. (1972) The influence of semicarbazide induced depletion of GABA on presynaptic inhibition. *Brain Research.*, **43**, 161-169.

Chang, T.C. (115 B.C.) Suanzaorentang. In: *Chin-Kuei-Yao-Lueh.* (Chang, T.C., Ed.), p. 75, China.

Chen, H.C. and M.T. Hsieh. (1985) Clinical trial of Suanzaorentang in the treatment of insomnia. *Int. J. Drug. Ther.*, **7(3)**, 334-338.

Chen, H.C. and M.T. Hsieh. (1985a) Studies on the Suanzaorentang in the treatment of anxiety. *Psychopharmacol*, **85**, 486-487.

Everett, G.M. (1974) Effect of 5-hydroxytryptophan on brain levels of dopamine, norepinephrine and serotonin in mice. *Adv. Biochem. Psychopharmacol*, **10**, 261-262.

Fielding, S. and I. Hoffman. (1979) Pharmacology of anti-anxiety drugs with special reference to clobazam. *Brit. J. Clin. Pharmacol.*, 7, 7-15.

Fuxe, K.L. Agnatr and P. Bolme. (1975) The possible involvement of GABA mechanisms in the action of benzodiazepines on central catecholamine neurons, In: *Mechanism of Action of Benzodiazepine*. (Costa, E. and P. Greengand, Eds.), 45-62, Raven Press, New York.

Ganong, W.F. (1979) Neurophysiologica basis of instinctual behavior & emotions. In: *Review of Medical Physiology*, 9th ed, 182-184, Lange Medical Publications, Los Altos, California.

Glowinski, J. and L.L. Iversen. (1966) Regional studies of catecholamines in the rat brain. I, *J. Neurochem.*, **13(8)**, 655-669.

Gunther, S. (1982) Simultaneous determination of serotonin, 5-hydroxyindoleacetic acid, 3,4-dihydroxyphenylacetic acid and homovanillic acid by HPLC with electrochemical detection. *J. Neurochem.*, **38(3)**, 840-846.

Hamilton, M. (1974) *Anxiety in Medicine.* In: *An Advanced Anxiolytic, Lorazepam, in Clinical Use*, 1-16, Excerpta Medica, U.S.A.

Hsieh, M.T., H.C. Chen, P.H. Hsu and T. Shibuya. (1986) Effects of Suanzaorentang on behavior changes and central monoamines. *Proc. Natl. Sci. Counc. B. ROC*, Vol. **10(1)**, 43-48.

Hsieh, M.T., T. Shibuya and H.C. Chen. (1984) The effect of clobazam and diazepam on central GABAergic system. In: *Journal of the Taiwan Pharmaceutical Association*, **36(4)**, 187-195.

Lackovic, Z.M., A.L. Parenti and N.H. Neff. (1981) Simultaneous determination of femtomole quantities of 5-hydroxytryptophan, serotonin and 5-hydroxyindoleacetic acid in brain using HPLC with electrochemical detection. *European J. Pharmacol.*, **69**, 347-352.

Mabry, P.D. and B.A. Campbell. (1973) Serotonergic inhibition of catecholamine-induced behavioral arousal. *Brain Res.*, **49**, 381-391.

Rudolf, H.S. (1982) Neurotransmitters in anxiety. *Arch. Gen. Psychiat.*, **39**, 735-744.

Shibuya, T.K. Sato and B. Salafsky. (1982) Simultaneous measurement of biogentic amines and related compounds by high performance liquid chromatography. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **20(7)**, 297-302.

Stein, L., C.D. Wise and J.D. Belluzzi. (1975) Effects of benzodiazepines on central serotonergic mechanisms. In: *Mechanism of Action of Benzodiazepines*. (Costa, E. and P. Greengand, Eds.), 29-44, Raven Press, New York.

Wachtel, H. and N.E. Anden. (1978) Motor activity of rats following intracerebral injections of drugs influencing GABA mechanisms. *Naunyn. Schmeidberg's Arch. Exp. Path. Pharmak.*, **302**, 133-139.

Wallach, D.P. (1961) Studies on the GABA pathway, I. The inhibition of γ-aminobutyric acid-α-ketoglutaric acid transaminase *in vitro* and *in vivo* by U 7524 (aminooxyacetic acid). *Biochem. Pharmacol.*, **5**, 323-331.

M.T. Hsieh, H.C. Chen, H.C. Kao and T. Shibuya

酸棗仁湯對於大白鼠中樞Adrenergic與Serotonergic系統之影響

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摘　　要

中國古方酸棗仁湯是一種很有效的抗焦慮劑，著者等曾探討其作用機轉，顯示酸棗仁湯能降低中樞單胺之轉換速率及兒茶胺之活性。

本研究結果發現酸棗仁湯(1)能顯著地拮抗由5-HTP所誘發引起之運動量抑制現象。(2)能顯著地抑制由p-CPA所誘發引起之運動量興奮現象。(3)對baclofen, muscimol, AOAA和thiosemicarbazide等所誘發引起運動量之影響，在統計上無顯著的意義。(4)能顯著地降低VMA在腦部striatum及hippocampus處之濃度，HVA在腦部hippocampus處之濃度及5-HIAA在腦部striatum及hypothalamus處之濃度。(5)腦部striatum及hippocampus處由α-MT所產生DA濃度的降低，酸棗仁湯能顯著地將其反轉。(6)腦部amygdala處由p-CPA所產生5-HT濃度的降低，酸棗仁湯能顯著地將其反轉。

由以上實驗結果顯示，酸棗仁湯能够影響serotonergic的活性，但對GABAergic的活性並無顯著的影響，且其主要的作用位置可能是在中樞邊緣葉系統。